SUTHERLAND

An Histological Study of the Regenerating Nerve Cord of the Frog Tadpole

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AN HISTOLOGICAL STUDY OF THE REGENERATING NERVE CORD OF THE FROG TADPOLE

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THESIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

George Fred Sutherland

ENTITLED An histological study of the regenerating nerve cord of the frog tadpole.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Master of Arts

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I. STATEMENT OF THE PROBLEM.

The present paper is an histological study of the early stages of regeneration in the nerve cord of the tadpole of Rana clamitans. Fraisse (1885) studied these early stages in several vertebrates in order to discover the origin of the regenerated tissues. The following conclusions of his are substantiated in so far as they are touched upon by the present study of regeneration in the nerve cord.

"(1) Both in amphibians and reptiles, injured tissues can produce only new tissue like themselves. The leucocytes assume only the function of nutrition and of devouring the broken down parts of tissues. They never become fixed tissues,- neither connective tissue nor any other sort.

(2) All the tissues are capable of regenerating themselves, either out of their differentiated elements or out of a matrix. As a matrix for the epidermis there is the Malpighian layer of the skin; for the central nervous system, the epithelium of the central canal of the nerve cord; and for the musculature, the spindle fibers."

There remains the further problem of the mechanism by which the old organs present at the cut surface replace their lost parts. There are two distinct stages in this process of regulation, (1) degenerative and (2) regenerative. First the injured cells at the cut edge degenerate. Then follows regeneration proper, or the formation of the new organ from the remaining elements of the old.

There are three ways in which regeneration proper might take place. (1) The cells at the cut edge of each organ by dividing might extend outward, and in time form the completed organ: (2) the cells in front of the cut edge might wander backward; and (3) the cells in front of the cut edge might divide in situ and push backward the more distal cells. These possible methods of regeneration will be made clearer by a diagram of that part of the hollow neural tube extending forward from the cut,(fig. 1, Plate I). If (1) (division of cells at the cut edge) were the method of regeneration, we should find after the operation that the cells at the cut surface A, or from A to C, are dividing rapidly, while from C to B about the normal number of cells is dividing. If (2) (migration of cells) were the method, we might find no dividing cells at all, but should expect to find that the cells from B to A or possibly only from D to A are turned with their long axes parallel to the longitudinal axis of the nerve cord as if moving toward the cut end. If (3) (division in situ) were the method, we should expect to find dividing cells all the way from B to C or possibly concentrated in a growing Zone ED.

The present work aims to give an account of the histological changes, both degenerative and regenerative, involved in the origin of the regenerated nerve cord, and especially of the mechanism by which the new organ is formed from the old. The study is based primarily on nuclear phenomena.

II.MATERIAL AND METHODS.

The frog tadpole was chosen for this work because it is easily kept alive in the laboratory, it regenerates readily and its tail is comparatively simple in histological structure. Since histological changes cannot be watched in the live animal, it is necessary to make serial sections of tails after various intervals. This enables one to follow the process of regeneration from stage to stage. But to get uniform results from this method and eliminate individual variations, one must take tadpoles as nearly alike as possible at the start, operate on all at the same time, keep them under uniform laboratory conditions and make sections of several individuals at each stage.

On October 12,1913, seventy frog tadpoles, varying in length from 30 to 60 mm., and of the species Rana clamitans, were brot into the laboratory. Two days later they were put into individual finger bowls each containing approximately the same amount of water. Forty-four medium sized individuals (32 to 40 mm. in length), chosen to constitute the main series, were grouped by 2's or 3's. Those of each group were as nearly alike as possible and each group was treated as a unit in the time of operation, killing, &c.. The extremes, both larger and smaller than the main series, were given the same treatment but were not used except where the main series was lacking. None of the tadpoles was fed during the course of

the experiment and none died from the effects of laboratory conditions.

On October 15, the first operations were performed. Each tadpole was transferred from the finger bowl to a paraffin block and approximately one fourth of the tail was removed, with a sharp scalpel, at right angles to the plane of the tail. The animal was returned to the finger bowl and the removed part put into Gilson's killing fluid. At the end of the period of regeneration, the animals were again taken out onto the block and the regenerated tail plus a second fourth of the normal tail was removed and put immediately into Gilson's killing fluid. The times of killing were as follows: normal, immediately after the operation, 1, 3, 5½, 9½, and 14 hours, and 1, 2, 3, 4, 6, 8, 9, 10, 12, 14 and 16 days after the operation. Usual methods of technique were followed. Delafield's haematoxylin and acid fuchsin stain the nuclei blue and the cytoplasm pink but do not distinctly bring out the cell boundaries. For the most part, sections were made in the sagittal plane.

III. OBSERVATIONS.

Histology of the Normal Nerve Cord.

The study was confined to the histology of regeneration in the nerve cord, since a preliminary examination showed that this organ of all those in the tail was best adapted for a study of the present problem. Figure 2 (Plate I) shows by a sagittal section the nerve cord and its relation to the surrounding

tissues. Figure 3 (PlateI) shows a transverse section of the nerve cord alone. It is a hollow tube which distally is formed of a single layer of cells.The nuclei are very near the inner border of the cells so that there is a wide outer zone of cytoplasm but practically no inner cytoplasmic zone. At this stage in the development of the tadpole, the cells near the distal end of the nerve cord show little differentiation. More proximally there is more than one layer, but the outer layers are formed by division and migration of cells from the layer lining the central canal, (Fraisse). The whole nerve cord including ganglia differentiates from the cells of this lining epithelium.

Degenerative Changes after an Operation.

When a tadpole's tail is removed the old notocord extends out beyond the other tissues, and the connective tissue between the notocord and nerve cord is usually broken so that the nerve cord bends dorsally as in figures 7 and 8 (Plate II). A transverse cut thru the tail leaves the various organs at the cut surface in contact with the surrounding medium, the water in which the tadpole lives. Sections of tadpoles killed immediately after the operation show the direct effect of the cutting,(figs. 4 & 5, Plate I).Many nuclei and cells are broken and irregular in appearance and may be loosened or torn apart from each other. The injured nuclei at the cut edge and extending forward with decreasing frequency, are homogeneous in appearance and

take a deep haematoxylin stain. Undoubtedly some of the nuclei are cut, and this accounts for the irregularity in shape of a good many. But a good many others, also staining deeply, are rounded and smaller than normal nuclei. These may be either normal nuclei which under the stimulus of the operation are contracted or compressed,or cut nuclei which have rounded off. These deeply staining nuclei, whether rounded or irregular in shape, are smaller than normal nuclei, so it may be that the chromatin, which stains deeply,is condensed on account of the loss of achromatic material.

The same assumption is borne out by the somewhat different appearance of nuclei in the tadpoles killed one hour after the operation,(fig.6, PlateI).Some are rounded as before; others are angular or slightly hour-glass shaped, with rather dense cytoplasm extending out from the corners. If parts of the nuclear membrane were held by the cytoplasm while the nucleus as a whole decreases in volume either by contraction or loss of achromatin, the nuclei might present such an appearance. Moreover there are gradations from hour-glass-shaped to normal nuclei and corresponding gradations in size and depth of stain. In cases of this sort there are often vacuoles or cytoplasm between the nuclei as if they had shrunken, whereas in the normal cord, the nuclei are so close together that no cytoplasm can be seen between them. These facts indicate that normal nuclei become darkly staining nuclei by contraction or by loss of achromatic material.

This "contraction" of nuclei seems to be caused by contact with the water or the killing fluid, or the succession of the two, as well as by direct injury from the scalpel, for other nuclei which are in contact with the exterior only thru the central canal show this phenomenon. In some cases, the end of a nucleus nearest the central canal is deeply stained and contracted while the other part is normal, (fig. 4, Plate I). The question immediately arises, why does not the water or other external factor enter the open neural tube and cause the contraction of the inner parts of practically all nuclei in the nerve cord? It may be due to the presence in the nerve cord of some substance which prevents the ready admission of external fluids. Since the sections show very little structure within the central canal, this content must be liquid or semi-liquid. However, in a number of sections there is a rather long narrow band of cytoplasmic material which may be the more solid part of a semi-liquid substance contracted by the killing reagent. There are other evidences of the presence of such a liquid. The sections from two of the tadpoles killed one hour after the operation, show a coagulation of the outer surface of the blood plasma covering the wound, but over the nerve cord this coagulating process is delayed. The most plausible explanation seems to be that some cerebro-spinal fluid,(compared by Barfurth to the cerebro-spinal fluid of mammals,) exerts an outward pressure which breaks thru any slight hardening of the plasma at this point. Perhaps transference of the animal to a medium

of different density, the killing fluid, aids the outburst. Sections of another tadpole killed at one hour, show the presence of this coagulated plasma over the end of the nerve cord as well as over other parts of the tail.

The outward pressure of a fluid would tend to push out into the blood plasma any free elements such as the injured and degenerating nuclei with very little cytoplasm and hence little connection with other cells; and when this fluid breaks thru, some of these nuclei may break off and float away. At one hour after the operation, broken and small rounded nuclei are seen in the end of the nerve cord and extending out into the hardened layer of the plasma, giving evidence of some force acting outward at this time,(fig. 7 , Plate I). Other evidences will be mentioned in describing the stages at which they appear.

Three hours after the operation there are fewer of the angular nuclei than at one hour and more of the round deeply-staining nuclei. The latter vary in size from that of similar ones in the earlier stage down to fragments. Moreover some of the larger of these seem to be in the process of fragmentation, that is, stages indicating direct division are seen. The gradation in size and depth of stain at one hour from normal nuclei nearly to rounded ones, and the gradation down to fragments at three hours, as well as the appearances of fragmentation, make it fairly clear that normal nuclei just in front of the cut edge may contract, become rounded and fragment. This must be a degenerative

process.Even finer intermediate steps are seen in preparations of later stages.

Sections of one individual at this period appear very much like those immediately after the operation. The darkly staining nuclei are similar, and the nerve cord is not covered either by epidermis or plasma, so that a recent outbreak of the cerebro-spinal fluid must have taken place. In this case a second contact with the exterior has again started the degenerative process.

At five and a half hours, the nerve cord is entirely covered by the thickened plasma layer in which is a group of fragmenting globular nuclei. In one preparation at this stage, the epidermis has closed-in over the entire wound. There is a series of stages in the degeneration of nuclei. Some are only slightly smaller and darker than normal nuclei, others have the angular appearance characteristic of nuclei one hour after the operation, while still others are round and fragmenting. At this stage there is another evidence of the presence of a cerebro-spinal fluid. The plasma covering the end of the nerve cord is pushed outward, making a knob-like extension of the central canal similar to that shown in figure 8, (Plate II). This did not appear in earlier stages either because not enough cerebro-spinal fluid was present, or because the plasma layer had not coagulated sufficiently to resist the outward pressure of this fluid.

Of the two preparations of tadpoles killed after

a nine and a half hour interval, one shows the epidermis and plasma covering all the wound except the neural tube; the other shows this part also covered. In the former, the sides of the neural tube are separated as if by a recent outburst of cerebro-spinal fluid, and deeply-staining rounded and fragmenting nuclei are seen. In the second preparation, the deeply-staining nuclei are all small and fragmentary. In other words no more nuclei seem to be starting to degenerate.

At fourteen hours, plasma and epidermis cover the nerve cord tho the plasma is pushed outward by the cerebro-spinal fluid, (fig.8, Plate II).There are nuclear fragments in the cord, and degenerating nuclei in the plasma. Another preparation at the same time shows the nerve cord still open to the exterior as well as the nuclear appearance of an earlier stage.

At twenty-four hours, only a few of the nuclei are slightly smaller and darker than the normal. At this time there appear near the end of the nerve cord, granular leucocytes containing pigment granules and fragments which closely resemble the fragments of degenerating nuclei. It may be that the leucocytes appear at this time and dispose of nuclear fragments. After one day, the degenerating nuclei are too rare to be significant.

The degenerative process which the forgoing facts seem to show,may be indicated diagrammatically as follows:

Cells directly cut ⟶ broken nuclei ⟶ rounded nuclei ⟶ fragments ⟶ disposed of by outbreak of cerebro-spinal fluid, or by leucocytes.

Cells just in front of those cut ⟶ angular nuclei ⟶ rounded nuclei ⟶ fragments ⟶ disposed of by leucocytes.

Enlargement of Nuclei.

A few preparations of the nerve cord soon after the operation show plainly that the nuclei near the end, but just in front of the deeply-staining nuclei, are larger than those of the normal cord. The long axes of nuclei close to the edge were measured and compared to nuclei of the same preparation which are some distance forward in the old tissue, (Table 1). Immediately after the operation and in the very early regeneration stages, the nuclei near the end are larger, but the difference decreases until after 9½ hours it is hardly significant. This enlargement might be preparatory to normal division or it might be a swelling which is a degenerative change preliminary to fragmentation. Since this size difference is greatest at the very beginning and decreases during the first day until it is no longer significant, and since mitotic divisions are not seen in numbers until the third day, the enlargement is probably an early stage in nuclear degeneration.

TABLE I.

Time of Regeneration	Nuclear Length Close to Edge	Nuclear Length in Front of Edge	Difference in Length
Normal	7.9	7.5	.4
Immediately	10.5	7.8	2.7
1 hour	11.3	9.1	2.2
3 hours	8.6	7.3	1.3
5½ hours	12.8	10.5	2.8
9½ hours	8.1	8.0	.1
14 hours	7.6	8.3	-.7
1 day	8.2	6.6	1.6
2 days	8.1	8.0	.1
3 days	8.2	8.9	-.7
4 days	8.4	8.2	.2
6 days	10.7	10.4	.3

Explanation: Each measurement recorded here is the average of the measurements of 9 or 10 nuclei.

Temporary Partial Closing of the Nerve Cord.

After the degenerative process is complete and the deeply staining nuclei have disappeared, the end of the nerve cord starts to close over. By the first day, the nuclei in the end of the cord have begun to spread apart, stretching out the connecting cytoplasm, (fig.11, Plate II). In general they extend toward the opposite wall of the central canal, thus narrowing the opening at the end. Some sections show pseudopod-like cytoplasmic extensions of the cells into the central canal as if closing were to be produced by amoeboid movement of the cells. Figure 9 (Table II) shows a section thru one side of the nerve cord in which one layer of cells, not quite at the end, is extending down into the central canal. Up to about six days phenomena such as these may be seen, but sections from six to sixteen days show that the closing is not completed within that period. By sixteen days the new tail is almost as long as it will become (Durbin, 1909), and the nerve cord reaches back close to the epidermis at the posterior end. Still these later preparations show the sides of the neural tube gaping open, and red blood corpuscles extending forward into the central canal of the new cord, as if the pressure of the cerebro-spinal fluid is not sufficient to keep them out.

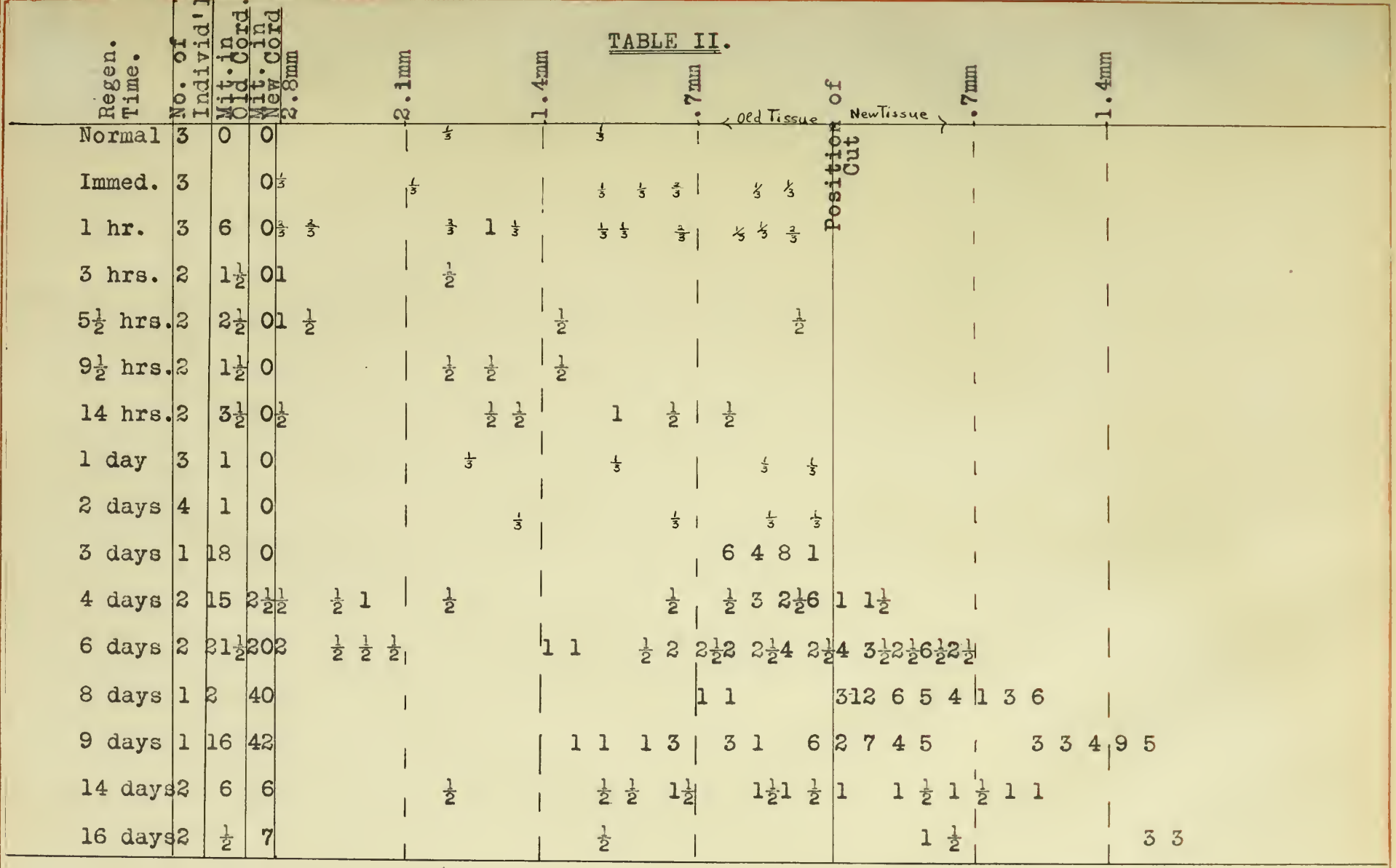
Cell Division.

In an organ such as the nerve cord in which the nuclei lie close together, it is difficult to determine an

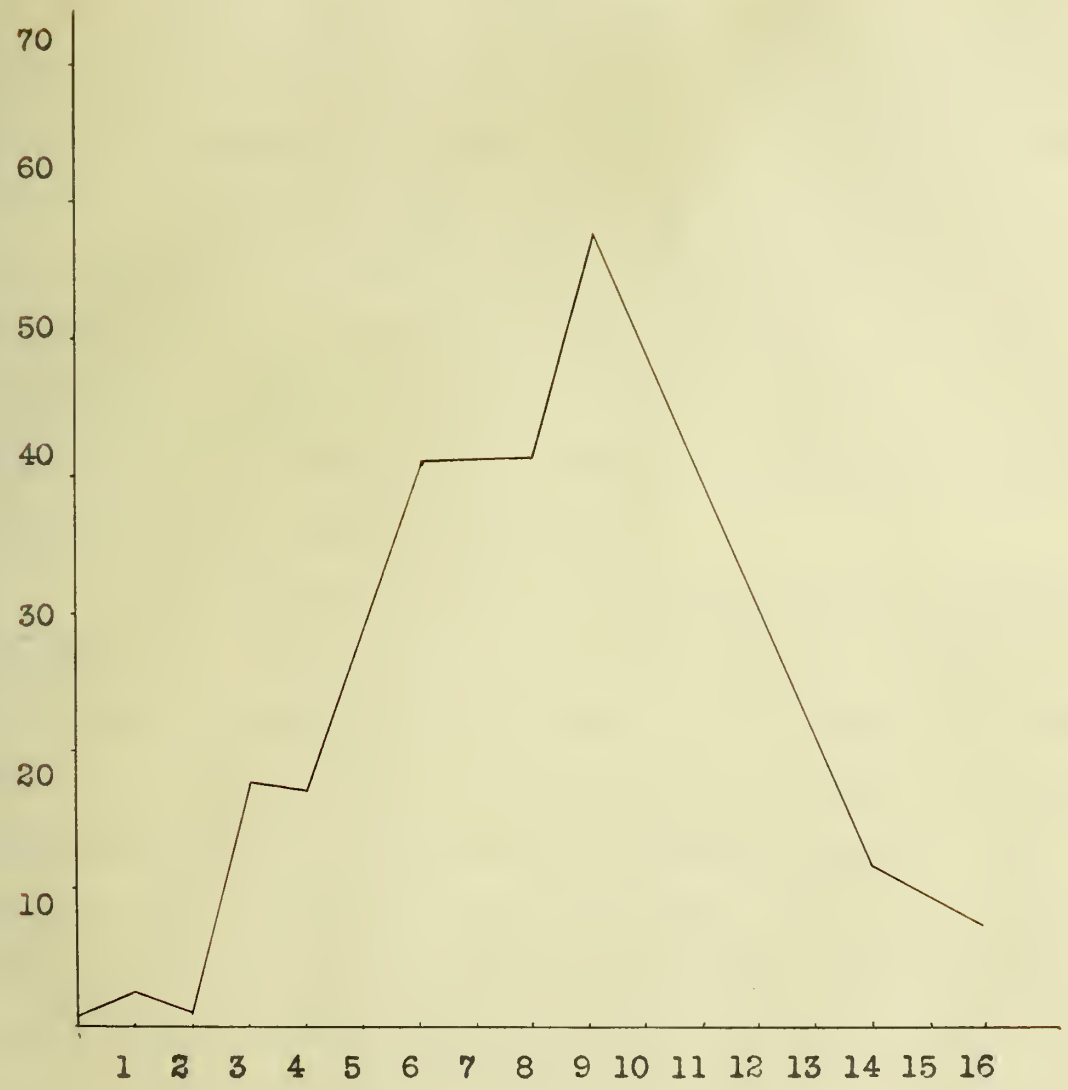
amitotic division. To be sure that amitotic divisions do occur, one must find continuous stages in nuclear and cellular constriction without the formation of chromosomes. Because of the massing of nuclei, this cannot be determined in the normal nerve cord. The present study gives no evidence that the normal nuclei divide amitotically, but stages in direct division can be seen in the deeply-staining nuclei at the cut edge. Is this amitosis or fragmentation? Do the daughter nuclei form normal nuclei, or do they divide several times and degenerate? There is no definite evidence that nuclei which divide directly ever become normal again. But at successive stages the deeply-staining nuclei become smaller and smaller down to fragments, so that the direct division is probably a fragmentation as a part of the degeneration of injured nuclei.

Mitotic divisions can be easily distinguished by the chromosome formation. All the preparations were examined and the distance of each mitotic division from the cut edge was measured. The results are shown in Table II. In the sections of the normal tail the number of divisions is the smallest, but since up to three days the mitoses are scattered and the number of individuals small, there is no reason for considering these anything but normal. During this period of degeneration of injured nuclei, there are almost no mitotic divisions close to the edge. On the third day the nuclei just in front of the cut edge are proliferating rapidly;at four days there are a few divisions

TABLE II.



This table represents the position and number of mitotic divisions taking place at different times. In most cases the number given is the average of 2-4 individuals.



Text figure 1. This curve gives the number of mitotic divisions in that part of the nerve cord within 3mm. of the cut edge. The abscissa represents the period of regeneration and the corresponding ordinate gives the average number of mitoses found in the individuals killed at the end of that period.

past the cut; at six days there are almost as many divisions in the new nerve cord as in the old; at eight and nine days most of the divisions are in the new cord; at fourteen days there are scattered mitoses only, both in the old and new cord, and at sixteen days most of the dividing cells are in the very end of the new cord. If later divisions follow this general trend it seems likely that the rest of the nerve cord will be formed by a growing zone at the tip, and until the new cord is complete the number of mitoses near the tip would probably decrease gradually.

Text figure one gives the average number of mitoses in the nerve cord at each stage, and therefore represents the rate of growth at these times. On the second day there is a considerable mass of tissue over the whole wound, tho only degenerative changes have been taking place in the nerve cord. Beginning about this time, the nuclei in the end of the cord loosen and draw apart somewhat stretching out the cytoplasm between them, ~~and~~ (fig. 11, Plate II). This is apparently the first extension in length of the nerve cord. At three days, active proliferation of cells has begun but the pulling apart or stretching toward the cut edge continues. Figure 10, (at six days) shows the cells in one part of the cord stretched out to such an extent that vacuoles are left between the cells. It is during the period from four to sixteen days that most of the increase in length takes place, by active proliferation and migration of cells.

IV. DISCUSSION.

General Considerations.

Regeneration has been considered as a morphological and physiological rejuvenation. Morphologically, the cells which form the new organ may be considered embryonic, for they have large nuclei and comparatively little cytoplasm. Physiologically, these cells may be considered embryonic, for they are dividing rapidly. As cells become old, they accumulate cytoplasmic material, become specialized and cease rapid division. Child, who has studied ~~rejuvenation~~ regeneration from the physiological or dynamic point of view, has shown that in Planaria dorotocephala, cells physiologically isolated from the dominant head region either in normal development or by an operation, become embryonic and form a new individual.

One may watch the growth of an embryo and follow the formation of the various organs, but unless he can in some degree control the process, at least change it by changing the conditions surrounding the embryo, he cannot fully grasp the meaning of the process which he is watching. The ease of influencing regeneration and the similarity of regenerative development to normal embryonic development offers the experimentalist material for the study of the factors influencing growth and differentiation in general. An histological study of the regenerative process is valuable not only because it helps explain the macroscopic changes, but because it adds to our knowledge of the principles of tissue differentiation.

A number of workers have studied the histology of regeneration. Fraisse (1885) and Barfurth (1891 and 1903) used

vertebrate material. The chief interest in this earlier work was the origin of the new tissue with special reference to the germ layer idea. In most cases an organ differentiates from tissue of the same layer in regeneration as in normal embryonic development. The present study confirms this result so far as the nerve cord is concerned. But in lumbriculus and certain naids, the pharynx develops from ectoderm in embryonic development and from endoderm in regeneration. As Morgan (1901, p 193) says, "This result has an important bearing on our ideas concerning the value and meaning of the so-called 'germ layers', and has helped to bring about a revolution of current opinion as to the importance of these layers." The origin of tissues in regeneration when studied from the point of view of origin alone has largely lost interest, but is of intense interest when studied from the standpoint of tissue differentiation.

There are obvious disadvantages in studying a process from stained sections, as is necessary in working out problems of this sort. The appearances at successive stages can be studied and drawn, but the sequence of events, and especially the explanation of the process is more difficult. In the present paper the facts have usually been given in the order in which they occur, but when some inference, founded on appearances at later stages, seemed to require more substantiation before being used as a basis for the description, these appearances have been described beforehand. In general, the observations presented in the present paper substantiate the facts presented by Fraisse and Barfurth.

Amitosis versus Fragmentation.

Fraisse in describing the regulative process at about two days after the operation, says," Considerable outwandering of leucocytes takes place at the cut edge, and it is these especially which account for the formation of the homogeneous, lymph like border which first covers the wound. The nerve cord in my sections now extends out close to this homogeneous border and the elements which compose it always separate from each other along this border even after twenty four hours. Then a considerable proliferation of nuclei occurs. The elements are strongly stained by picro-carmine, and now one sees taking place in them,- near to similar large corpuscles- nuclear divisions without even a trace of a mitotic figure. The nucleus constricts in the usual way in the form of a shoe sole, and similar elements come from each half. I think I can observe not only a single constriction of the nucleus, but many, so that by this process the nucleus can be divided not only into two but into more parts."*

Fraisse discusses further the evidence that the nuclei from the end of the nerve cord, which are found in the lymph-like border, divide amitotically. This agrees with the present observations but he is satisfied to show that direct division does take place. So far as my preparations show, there are few evidences that the nuclei which divide amitotically afterward become normal nuclei. In some of the preparations of stages at which the darkly staining nuclei have almost disappeared,

* References to Körnern or nuclei of the gray substance, which are not present in the distal region of the nerve cord, have been omitted.

there are few nuclei which stain only slightly darker than the normal nuclei, and at this time there are no stages between these and the fragments. These few slightly darkened nuclei may, then, be forming normal nuclei again. All other evidence points toward the conclusion that at successive stages, these darkly staining nuclei become smaller and smaller as if fragmentation, or repeated direct division, is taking place. The conclusion from these facts is that nuclei which have only started to degenerate may perhaps return to the normal condition, but that nuclei which have gone so far as to divide directly are destined to fragment.

The Appearance of Leucocytes.

Barfurth, working on the regenerating nerve cord of the frog larva at forty six hours and at three days, gives the following: "The terminal part of the regenerating medullary tube shelters within and between its epithelial cells many fatty degenerating leucocytes. Many large and small fat drops which are here everywhere, I have traced back in their origin to such disintegrating wandering cells. Moreover many pigment granules are here also, which arise by regressive metamorphosis of disintegrating leucocytes, (pigment degeneration)."

Barfurth figures a nerve cord of a larva of Triton cristatus after the sixth day of regeneration, which these leucocytes and fat drops are shown. His figure is very similar to figure 11, which shows a section of a tadpole killed twenty four hours after the operation. Both Fraisse and Barfurth mention particularly the presence of leucocytes in the early regeneration stages, but in the present study, leucocytes were

not found in large numbers. Up to the end of the first day, none at all were seen close to the nerve cord. The earliest stage mentioned by Barfurth is that after a forty six hour regeneration period, and this probably accounts for the different interpretation he gives of the origin of the "fat drops" or fragments. If these fragments are followed back into earlier stages in my sections, they become larger and larger and are seen to be identical with the degenerating nuclei. To be sure, the leucocytes when they first appear in the nerve cord region contain "fat drops", but is it not more reasonable to suppose as Fraisse suggested, that the leucocytes which are present at this time dispose of the fragments of injured nerve cord nuclei?

Temporary Closing of the Nerve Cord.

Barfurth describes the closing of the nerve cord at three days by means of cytoplasmic extensions of the cells, such as were seen in the present preparations. Then "the cerebro-spinal fluid, accumulating again, presses on the newly formed, little resistent part of the tube and forces it knob-like outward. The cells fit themselves to this pressure and remain in this position for sometime." In his summary Barfurth mentions this as a temporary closure of the nerve cord, so his later preparations evidently show the cord again open. The regenerated nerve cord at sixteen days has almost reached its maximum lebgth but it is not yet closed. The interesting question arises, is the completely regenerated nerve cord open at the end or closed as in the normal tail? This question does not come within the scope of the present study.

Rate of Division. Amitosis versus Mitosis.

Durbin (1909), in analyzing the rate of increase in length throughout the regenerative process in the tail of Rana clamitans, distinguishes four periods. "The operation was followed by an interval of low rate, succeeded of rapidly increasing rate, then by one of rapidly decreasing rate and finally an interval gradually approaches zero. The first low period is explained by a combination of two factors, (a) the shock of the injury and (b) the formation of a cap of embryonic cells which is to serve as a basis for the more active regeneration. The second or period of rapidly increasing growth is the one in which practically all the cells in the new part are undifferentiated and rapidly dividing. The third and fourth periods are explained by the appearance of differentiation, which lessens the number of dividing cells."

Text figure one, based on the number of mitotic divisions in the nerve cord, shows these same periods. The initial period of low rate covers the first two days; that of rapidly increasing rate includes the third to ninth day; the period of rapidly decreasing rate extends from the tenth to sixteenth days, and the period of gradually decreasing rate, tho not covered in the present work, would undoubtedly extend on from about sixteen days. In the light of this histological study, a somewhat different interpretation might be given to the initial period. It is during these first two days that degeneration of the injured cells is taking place. Tho at this time a cap of undifferentiated cells is being formed over the wound, the nerve cord does not participate in the formation of this cap,

nor is any such cap formed at the end of the nerve cord. Since the nerve cord cells in this part of the tail are so slightly differentiated, the new cord is formed from the old without the separation of a group of special embryonic cells.

The similarity of the rate curves based on a counting of the mitotic divisions with that based on the amount of tissue formed at each period, seems to be significant. It shows that the rate of tissue formation is closely correlated with the number of mitotic divisions. This is indirect evidence supporting the view that amitotic divisions is not important in the formation of an organ by regeneration.

V. SUMMARY.

1. The regenerating nerve cord of the frog tadpole has been studied histologically in order to learn the mechanism by which the new cord is formed from the old.

2, During the first day after the operation, injured nuclei in the end of the nerve cord degenerate. There is first a contraction or loss of achromatin, and then a fragmentation of these degenerating nuclei. The fragments may be carried away either by the outbreaking of a cerebro-spinal fluid or by leucocytes which appear at this time. These fragments are parts of disintegrated nerve cord nuclei and not of leucocytes.

3. From two to six days there is a temporary partial closing of the nerve cord probably by migration of the cells near the end.

4. The cells of the old cord near the cut edge, by mitotic division and migration, form the new cord.

5. Durbin's analysis of the rate of regeneration, based on the measurement of length, is substantiated by a counting of mitotic divisions.

6. The number of mitotic divisions found at different period is proportional to the rate of regeneration at those periods. Amitotic division, if it occurs, is not important in the formation of the regenerated organ.

7. There is no direct observational evidence that amitotic division occurs in normal nerve cord cells.

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This work was carried on under the direction of Dr. Charles Zeleny. His suggestion of the problem, and constant interest in its progress are sincerely appreciated.

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VI. EXPLANATION OF FIGURES.

Figure 1. Diagram, explained in the text, p.2.

Figure 2. Sagittal section thru a part of the normal tail, showing the nerve cord and its relation to the surrounding tissues. n.c.-nerve cord, c.c.-central canal, n.t.c.-notocord, conn.t.-connective tissue, p.c.-pigment cell. (330 diameters.)

Figure 3. Transverse section thru the normal nerve cord, showing the nuclei and the outer cytoplasmic zone. c.c.-central canal. (890 diameters.)

Figure 4.-Transverse section thru the nerve cord immediately after the operation. c.c.-central canal, n.n.-normal nuclei, d.n.-darkly staining nuclei. (920 diameters.)

Figure 5. Sagittal section thru the side of the nerve cord immediately after the operation, showing the darkly staining nuclei at the cut end. d.n.-darkly staining nuclei. (920 diameters.)

Figure 6. Sagittal section thru the nerve cord one hour after the operation. This shows the "contracting" nuclei. c.c.-central canal, d.n.-darkly staining nuclei, n.n.-normal nuclei. (920 diameters.)

Figure 7. Sagittal section thru the nerve cord and surrounding region ~~nuclei~~ one hour after the operation, showing irregularly shaped, darkly staining nuclei in the end of the nerve cord and extending out into the coagulated plasma layer. d.n.-darkly staining nuclei, p.l.- plasma layer, conn.t.-connective tissue, n.t.c.-notocord. (330 diameters.)

Figure 8.-Sagittal section thru the end of a nerve cord fourteen hours after the operation. This shows the epidermal layer. the plasma layer, and the knob-like extension of the central canal, caused by the outward pressure of the cerebro-spinal fluid. Ep.-epidermis, c.c.- central canal, p.l.-plasma layer, n.t.c.-notocord. (330 diameters.)

Figure 9. Sagittal section close to the side of the central canal, showing a row of cells, not quite at the end, entending across the central canal. Other sections of the series show that the end of the cord is still open. c.c.- central canal, r.b.c.-red blood corpuscles, w.b.c.-leucocyte. (920 diameters.)

Figure 10. Sagittal section thru the new nerve cord six days after the operation. b.v.-blood vessel, mit.- mitotic figure, n.t.c.-notocord, conn.t.-connective tissue. (330 diameters.)

Figure 11. Sagittal section thru the nerve cord 1 day after the operation, showing the granular leucocytes at the end of the cord, and the pulling apart of nuclei in the lower part of the cord. c.c.-central canal, w.b.c.-leucocytes.(920 diameters)

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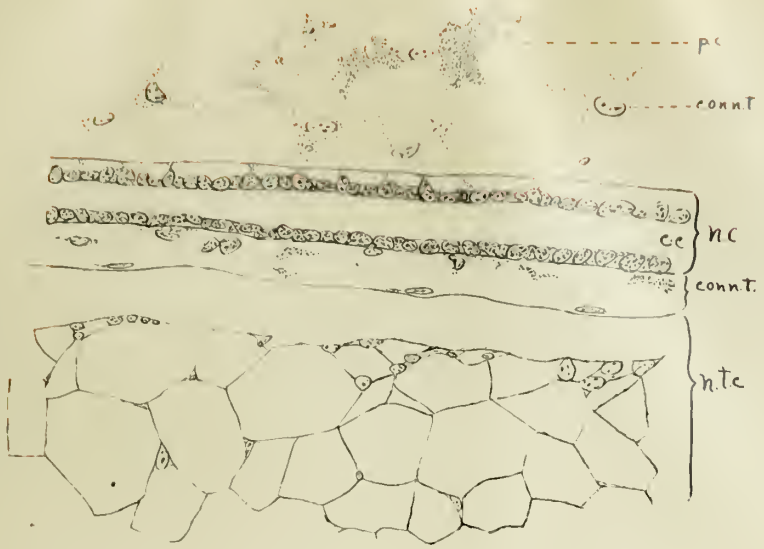

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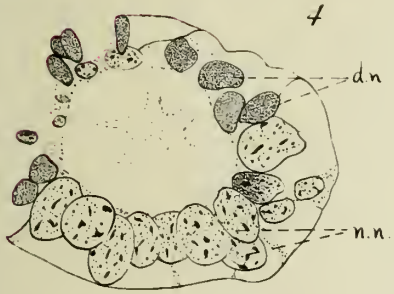

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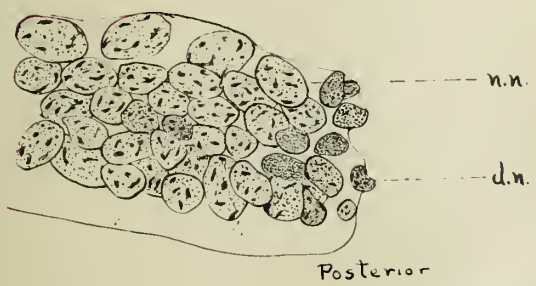

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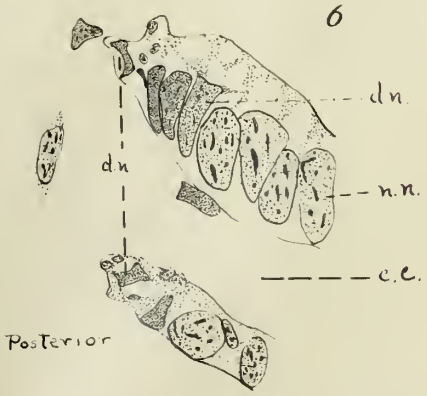

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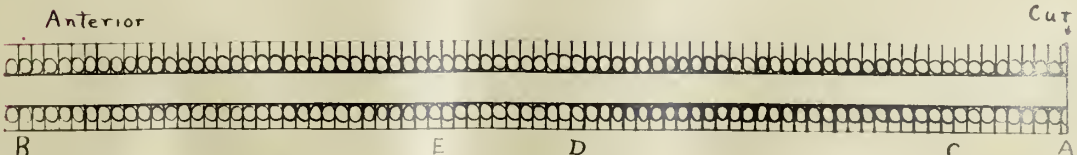

Plate I

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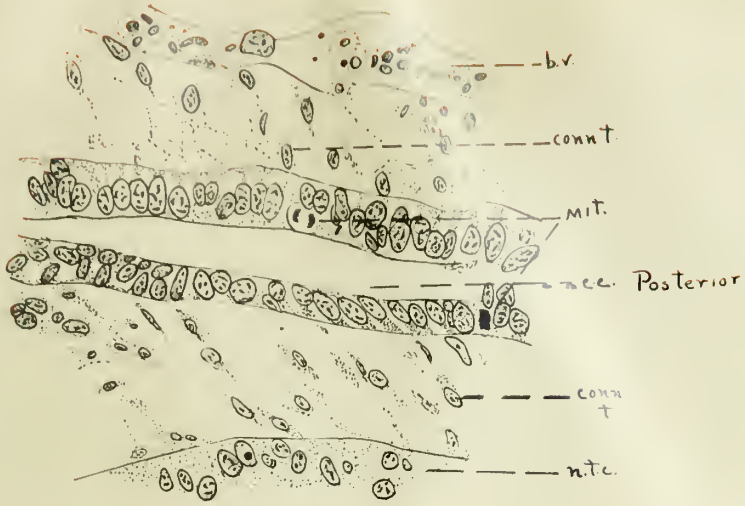

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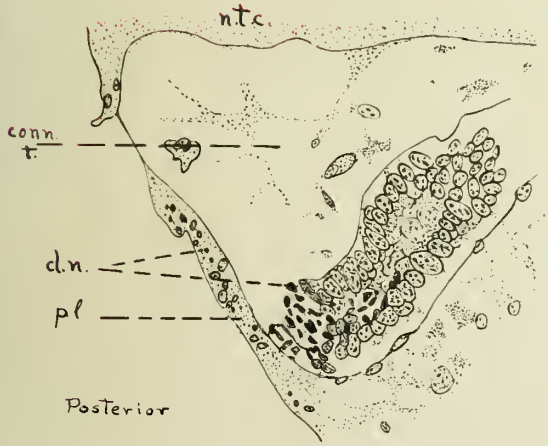

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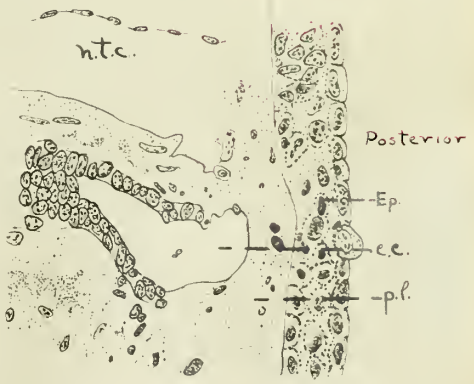

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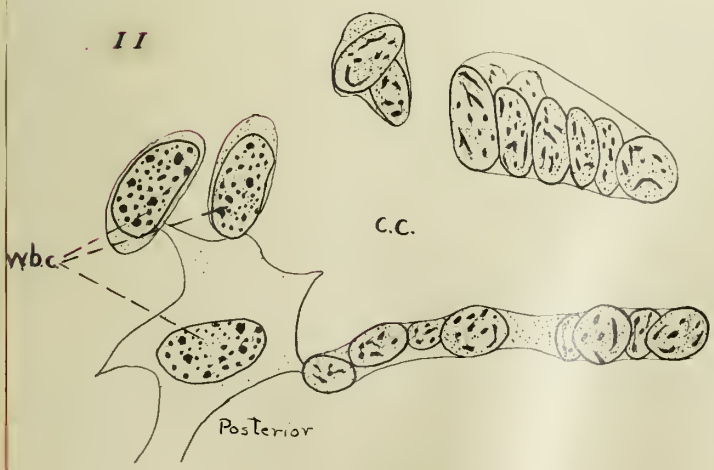

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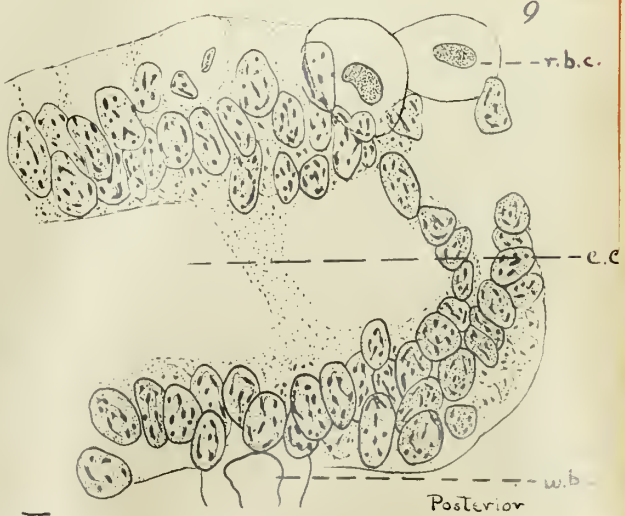

Plate II